
DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the enzyme sensor which uses the quantum method of the amount of chemicals, and this, and its manufacture method.

[0002]

[Description of the Prior Art] In recent years, in the medical field, the chemical sensor which carries out the quantum of the organic substance selectively is demanded. For example, in a clinical laboratory test, the quantitative analysis of a patient's **** and the urea in body fluid is important, when diagnosing a kidney function. Moreover, in case artificial dialysis is given to the patient of chronic renal failure, also when giving the count of artificial dialysis, and the rule of thumb of dialysis time amount and performing planned artificial dialysis, the quantitative analysis of a urea is indispensable.

[0003] Until now, according to reference 1: "the volume biosensors and for Shuichi Suzuki and Kodansha scientific ** (1984)", the attempt using the molecular recognition function which excelled [section / of a chemical sensor / recognizing-ability] in the enzyme is made, for example. The enzyme sensor which changes and carries out the quantum of the matter fluctuated when an enzyme recognizes the specific amount of chemicals (it is also hereafter called a substrate) and carries out the catalyst of the specific reaction to the amount of chemicals to an electrical signal with an oxygen electrode, a hydrogen peroxide electrode, a hydrogen electrode, an ammonia electrode, the carbon dioxide electrode, an ion-selective FET electrode, etc. is proposed.

[0004] As an example of the conventional enzyme sensor, an urea sensor is briefly explained with reference to a drawing. Drawing 9 is a cross section with which explanation of the conventional urea sensor is presented. This urea sensor is NH4+ which carried out decomposition generation from the urea by the urease 28 fixed to the porous glass film 12. The quantum is changed and carried out to the electrical signal with the silver-silver chloride electrode 24 through the ammonia permselective membrane 26.

[0005]

[Problem(s) to be Solved by the Invention] However, for example, in the urea sensor mentioned above, it was the structure which reaches an electrode (silver-silver chloride electrode) first after spreading a substrate (in this case, urea) in an enzyme fixed film and diffusing the film (ammonia permselective membrane) with which the product (NH4+) by the enzyme reaction of a there penetrates that product selectively further. For this reason, there was a defect of requiring time amount until the electrical signal

outputted from an urea sensor became constant value, after starting measurement.

[0006] For this reason, the artificer concerning this application noted that the oscillation frequency of a quartz resonator, the oscillation frequency of a surface acoustic wave device, or resonance frequency decreased in proportion to the mass of the matter adhering to these quartz resonators or a surface acoustic wave device, as a result of repeating various examination. (A quartz resonator and a surface acoustic wave device are hereafter called vibrator collectively.) Moreover, oscillation frequency and resonance frequency are collectively called vibration frequency. Such a phenomenon is indicated by reference I: "living thing physics, vol.28, No.6 (1988)." According to the reference I, it is shown by by using a bimolecular membrane coat quartz resonator that sensing of the smell matter and the bitterness matter is possible.

[0007] Then, this artificer found out the method of carrying out the quantum of the amount of a substrate, without passing like a diffusion fault by fixing an enzyme to vibrator and detecting change of the vibration frequency of this vibrator, as a result of repeating various experiments and examination further.

[0008] The 1st object which this invention is made in view of such a point, therefore starts this application is to offer the quantum method of the amount of chemicals which can carry out the quantum of the amount of the amount of chemicals in a short time. Moreover, the 2nd object concerning this application is to offer the enzyme sensor used for the quantum method of the amount of chemicals of the 1st object. Moreover, the 3rd object of this invention is to offer the manufacture method of the enzyme sensor of the 2nd object.

[0009]

[Means for Solving the Problem] In order to aim at achievement of this object, according to a quantum method of the amount of chemicals this invention, it is characterized by carrying out the quantum of the amount of chemicals by fixing an enzyme to a quartz resonator and comparing with oscillation frequency of a quartz resonator when the amount of chemicals has not adhered oscillation frequency at the time of making the amount of chemicals adhere on the surface of a quartz resonator by enzyme reaction into which this enzyme decomposes the specific amount of chemicals, respectively.

[0010] Moreover, oscillation frequency at the time of making the amount of chemicals adhere on the surface of a surface acoustic wave device by enzyme reaction into which an enzyme is fixed to a surface acoustic wave device, and this enzyme decomposes the specific amount of chemicals according to a quantum method of the amount of chemicals this invention, [whether oscillation frequency of a surface acoustic wave device when the amount of chemicals has not adhered is compared, and] Or it is characterized by carrying out the quantum of the amount of chemicals by comparing resonance frequency when the amount of chemicals makes it adhere to a front face of said surface acoustic wave device with resonance frequency of a surface acoustic wave device when the amount of chemicals has not adhered.

[0011] Moreover, in fixing an enzyme to a quartz resonator or a surface acoustic wave device in enforcing a quantum method of the amount of chemicals this invention, it is desirable to use covalent bond of an enzyme and glutaraldehyde.

[0012] Moreover, in fixing an enzyme to a quartz resonator or a surface acoustic wave device in enforcing a quantum method of the amount of chemicals this invention, it is desirable to use a specific binding reaction of avidin and a biotin.

[0013] Moreover, in fixing an enzyme to a quartz resonator or a surface acoustic wave device in enforcing a quantum method of the amount of chemicals this invention, it is desirable to use a specific binding

reaction of streptoavidin and a biotin.

[0014] Moreover, a quantum method of the amount of chemicals this invention uses and is suitable to make an enzyme into an urease and carry out the quantum of the amount of ureas.

[0015] Moreover, according to the enzyme sensor of this invention, it is characterized by coming to fix an enzyme to a quartz resonator.

[0016] Moreover, according to the enzyme sensor of this invention, it is characterized by coming to fix an enzyme to a surface acoustic wave device.

[0017] Moreover, it is desirable to perform immobilization with an enzyme, a quartz resonator, or a surface acoustic wave device in operation of an enzyme sensor of this invention using covalent bond of an enzyme and glutaraldehyde.

[0018] Moreover, it is desirable to use a specific binding reaction of avidin and a biotin for immobilization with an enzyme, a quartz resonator, or a surface acoustic wave device in operation of an enzyme sensor of this invention.

[0019] Moreover, it is desirable to use a specific binding reaction of streptoavidin and a biotin for immobilization with an enzyme, a quartz resonator, or a surface acoustic wave device in operation of an enzyme sensor of this invention.

[0020] Moreover, it is good to make an enzyme into an urease in operation of an enzyme sensor of this invention.

[0021] Moreover, in manufacturing an enzyme sensor of this invention, it is desirable to perform immobilization with an enzyme, a quartz resonator, or a surface acoustic wave device using covalent bond of an enzyme and glutaraldehyde.

[0022] Moreover, in manufacturing an enzyme sensor of this invention, it is desirable to perform immobilization with an enzyme, a quartz resonator, or a surface acoustic wave device using a specific binding reaction of avidin and a biotin.

[0023] Moreover, in manufacturing an enzyme sensor of this invention, it is desirable to perform immobilization with an enzyme, a quartz resonator, or a surface acoustic wave device using a specific binding reaction of streptoavidin and a biotin.

[0024] Moreover, in enforcing a manufacture method for an enzyme sensor of this invention, it is good to make an enzyme into an urease.

[0025]

[Function] According to this invention, the quantum of the amount of a chemical (substrate) can be carried out by fixing an enzyme in vibrator and detecting change of the vibration frequency of this vibrator, without passing like a diffusion fault.

[0026] The enzyme reaction which fixes an enzyme in vibrator and is shown in the following formula (1) and into which this enzyme disassembles a specific chemical (substrate) [0027]

[Formula 1]

$$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E+P \quad \cdots (1)$$

[0028] (However, E, S, ES, and P are the enzymes, the chemicals (substrate), enzyme-substrate complexes, and products of isolation respectively.) Moreover, k1, k-1, and k2 It is the rate constant of ES to the

generation reaction of an ES complex, the dissociative reaction, and a product, respectively. Make an enzyme-substrate complex generate, this chemical is made to adhere to vibrator, and it is characterized by carrying out the quantum of the chemical from change of the vibration frequency of the vibrator by adhesion of this chemical. If the coating weight of the chemical to vibrator becomes constant value when the enzyme reaction shown in the above-mentioned formula (1) will be in a steady state, without passing through the immobilization which the product by the chemical and enzyme reaction which carry out a quantum diffuses according to this method, the quantum of the amount of chemicals can be carried out from vibration frequency change of the vibrator in that event.

[0029] Moreover, since the covalent bond of an enzyme and a glutaraldehyde, the specific binding reaction of avidin and a biotin, or the specific binding reaction of streptoavidin and a biotin is used [according to the manufacture method of the enzyme sensor this invention] in fixing an enzyme to a quartz resonator, an enzyme is firmly combinable to a quartz resonator.

[0030]

[Example] Hereafter, with reference to a drawing, the example of the enzyme sensor used for it and its manufacture method is collectively explained to the quantum method list of the amount of chemicals of this invention. In addition, it does not pass over numerical conditions, such as the material of construction used in the following examples and its amount, the processing time, and processing temperature, for a suitable example, therefore this invention of be [it / what is limited to these conditions] is clear.

[0031] The 1st example of the 1st example explains the urea sensor using the quantum method and this and its manufacture method of the amount of ureas which comes to fix an urease to a quartz resonator as an enzyme as an example of the enzyme sensor which uses the quantum method of the amount of chemicals this invention, and this, and its manufacture method.

[0032] The so-called AT cut quartz resonator is suitable for the quartz resonator used in the 1st example. It is because the measurement error according to the difference in the temperature at the time of the quantum of the amount of chemicals since the frequency drift to 1. temperature change is small by using an AT cut quartz resonator is for being generated, and carries out things and 2. thickness slip vibration, so the advantage that the frequency drift to the amount coating weight of chemicals is remarkable is acquired.

[0033] Manufacture of a urea FET sensor (the 1)

(A) - (C) of drawing 1 is process drawing with which explanation of the urea sensor of the 1st example and its manufacture method is presented. In each drawing, it is illustrating about one enzyme typically.

[0034] First, how to fix an urease 40 to the front face of a quartz resonator 30 as an enzyme through a GURATARU aldehyde is described.

[0035] First, a quartz resonator is processed by the silane coupling agent, and the amino group is formed in a front face. In this example, it is immersed in this 1 volume % aqueous solution at a room temperature for 1 hour, using aminopropyl triethoxysilane (Chisso Corp. make) as a silane coupling agent. Then, a quartz resonator is washed by irradiating a 20kHz ultrasonic wave for 30 minutes in pure water, and excessive aminopropyl triethoxysilane is removed. Next, covalent bond is formed between aminopropyl triethoxysilane and the front face of a quartz resonator 30 by heat-treating a quartz resonator for 20 minutes under the temperature of 110 degrees C ((A) of drawing 1).

[0036] Next, covalent bond is formed between a glutaraldehyde and aminopropyl triethoxysilane by

immersing this quartz resonator in the glutaraldehyde aqueous solution of 1 volume % for 1 hour. Then, a quartz resonator 30 is washed by irradiating a 20kHz ultrasonic wave for 30 minutes in pure water, and an excessive glutaraldehyde is removed ((B) of drawing 1).

[0037] Next, a quartz resonator is immersed for 2 hours into a phosphate buffer solution with a pH [7.2] of 100ml which contains 1mg (product made from Funakoshi Pharmaceuticals) of ureases 40 for this quartz resonator 30. An urease 40 is fixed to a quartz resonator 30 through a glutaraldehyde in the meantime. An unreacted urease is removed by washing with the phosphate buffer solution of pH7.2 ((C) of drawing 1).

[0038] Manufacture of an urea sensor (the 2)

(A) - (C) of drawing 2 is process drawing with which explanation of the urea sensor of the 1st example and its manufacture method is presented. In each drawing, it is illustrating about one enzyme typically.

[0039] Next, how to fix an urease 40 to the front face of a quartz resonator 30 as an enzyme through avidin and a biotin is described.

[0040] First, a quartz resonator 30 is processed by the silane coupling agent, and the amino group is formed in a front face. In this example, it is immersed in this 1 volume % aqueous solution at a room temperature for 1 hour, using aminopropyl triethoxysilane (Chisso Corp. make) as a silane coupling agent. Then, a quartz resonator 30 is washed by irradiating a 20kHz ultrasonic wave for 30 minutes in pure water, and excessive aminopropyl triethoxysilane is removed. Next, covalent bond is formed between aminopropyl triethoxysilane and the front face of a quartz resonator 30 by heat-treating a quartz resonator 30 for 20 minutes under the temperature of 110 degrees C ((A) of drawing 1).

[0041] Next, in order to combine a biotin 34 with this quartz resonator 30, covalent bond is formed between the biotin long arm 36 and aminopropyl triethoxysilane by immersing this quartz resonator in the heavy carbonic acid buffer solution of pH8.0 containing the NHS-LC-biotin (trade name) (it is also called a biotin long arm the product made from Funakoshi Pharmaceuticals, and the following) 36 with a concentration of 0.1mg [/ml]. Then, a quartz resonator 10 is washed by irradiating a 20kHz ultrasonic wave for 30 minutes in pure water, and an excessive biotin long arm is removed ((B) of drawing 1).

[0042] Next, a quartz resonator 30 is immersed for 2 hours using the method of Vernon (Vernon) indicated by reference III: "THE journal OBU cel biology (TheJournal of Cell Biology) Vol.93, pp.981-986 (1982)" into a phosphate buffer solution with a pH [containing 1mg of ureases 40 which combined avidin D(trade name) (product made from Funakoshi Pharmaceuticals) 38 as avidin 38 /7.2] of 100ml. A biotin 34 and avidin 38 carry out a specific binding reaction in the meantime. An unreacted urease is removed by washing with the phosphate buffer solution of pH7.2 ((C) of drawing 1).

[0043] Through the above process, the urease 40 was fixed to the front face of a quartz resonator 30, and the urea sensor was manufactured. In addition, the same effect is acquired, even if the method of immobilization of an urease 40 is not restricted to these and it uses the specific binding of streptoavidin and a biotin.

[0044] It measured as follows about the property of an urea sensor, next the response characteristic of the urea sensor which comes to fix an urease 40 to a quartz resonator 30 as an enzyme through a glutaraldehyde.

[0045] Drawing 3 is the block diagram having shown the system used for measurement of the response characteristic of the urea sensor of the 1st example. The terminal 44 of the quartz resonator 30 which fixed the urease of an urea sensor 42 is connected with the oscillator circuit 46. The oscillator circuit 46 is

connected with the radionuclide generator (power supply) 48. Moreover, the outgoing end of 46 of an oscillator circuit is connected also with the frequency counter 50, and this frequency counter 50 is connected with the computer 52. It is immersed in distilled water 56 in a beaker 54 by the urea sensor 42.

[0046] In the condition that this urea sensor 42 was immersed in the phosphate buffer solution 56 of pH7.2, it is specified quantity ****** about a urea to this phosphate buffer solution. And after adding a urea into a phosphate buffer solution, the oscillation frequency (vibration frequency) of the quartz resonator after 5-minute progress is measured. And it asked for the vibration frequency change by reducing the vibration frequency before adding a urea from this vibration frequency. It is because vibration frequency was fully stabilized as it is such elapsed time so that it might mention later having measured vibration frequency after the 5-minute progress which added the urea into the phosphate buffer solution.

[0047] Drawing 4 is property drawing of an urea sensor measured by the system of measurement mentioned above. The horizontal axis of the graph of drawing 4 shows the amount of ureas (g/ml) applied to a phosphate buffer solution, and the axis of ordinate shows vibration frequency change (Hz) of the vibrator of an urea sensor. The straight line I in a graph connects what plotted both. it turns out with the increment in the amount of ureas that the vibration frequency of vibrator decreases at a rate of about 1 law so that clearly from the graph of drawing 4 . Moreover, change of such vibration frequency was not seen at all to other amounts of chemicals, such as a glucose. Therefore, it turned out that property with the urea sensor of this example sufficient as an urea sensor is acquired.

[0048] Next, the graph of the measurement result of aging of the output signal at the time of the urea sensor of the 1st example and the urea sensor of the example of a comparison being immersed in the phosphate buffer solution containing the ten to 3 g/ml amount of ureas at drawing 5 , respectively is shown. The horizontal axis of a graph shows the elapsed time (minute) from measurement initiation, and as an output signal, an axis of ordinate sets variation of oscillation frequency and output potential to 100, and shows the variation in each saturation state for it by the relative value. The curve II in a graph shows aging of the oscillation frequency of the urea sensor of the 1st example, and the curve III in a graph shows aging of the output voltage of the urea sensor explained in the conventional example as an example of a comparison. In the urea sensor of the 1st example, the variation of frequency reached the saturation value mostly within 1 minute to having required for the urea sensor of the example of a comparison for about 20 minutes that the variation of output voltage reaches a saturation value so that clearly from the graph of drawing 5 . Thus, the urea sensor which is an example of the enzyme sensor using the quantum method of the amount of chemicals this invention and it showed that the quantum of the amount of the amount of chemicals could be carried out in a short time.

[0049] Moreover, the variation of frequency reached the saturation value mostly within 1 minute similarly [in the urea sensor which fixed and manufactured the urease using the specific binding of streptoavidin and a biotin / that a glutaraldehyde was used].

[0050] The 2nd example of the 2nd example explains the urea sensor using the quantum method and this and its manufacture method of the amount of ureas which comes to fix an urease to a surface acoustic wave device as an enzyme as an example of the enzyme sensor which uses the quantum method of the amount of chemicals this invention, and this, and its manufacture method.

[0051] In the 2nd example of manufacture of an urea sensor, an urease is combined with a surface acoustic wave device instead of a quartz resonator using the same method as the 1st example.

[0052] It measured as follows about the response characteristic of an urea sensor, next the response characteristic of the urea sensor 60 which combined the urease 40 with the surface acoustic wave device 70 through the glutaraldehyde.

[0053] Drawing 6 is the block diagram having shown the system used for measurement of the response characteristic of the urea sensor of the 2nd example. This urea sensor 60 is connected with amplifier 62 and 64. A frequency counter (not shown) is connected between amplifier 62 and 64, and oscillation frequency is measured. It is immersed by the urea sensor 60 into the phosphate buffer solution 68 of pH7.2 in a cistern 66. In the 2nd example, the urease 40 is combined using what formed 1st and 2nd tolan DEYUSA 74 and 76 on ST cut quartz plate 72 as a surface acoustic wave device 70 on these tolan DEYUSA 74 and the quartz plate 72 between 76.

[0054] It is specified quantity ****** about a urea in this phosphate buffer solution 68 in the condition that the urea sensor 60 was immersed in the phosphate buffer solution 68 in this example. And after adding a urea into a phosphate buffer solution, the oscillation frequency of the urea sensor after 5-minute progress is measured. It is because the oscillation frequency of a surface acoustic wave device was fully stabilized [that such elapsed time measured oscillation frequency after 5 minute progress after adding a urea into a solution, and].

[0055] Next, the measurement result of the response characteristic of an urea sensor is shown in drawing 7 . The horizontal axis of the graph of drawing 7 shows the urea concentration in a solution (g/ml), and the axis of ordinate shows oscillation frequency (it is also hereafter called vibration frequency) change (kHz) of an urea sensor. The straight line IV in a graph connects the plot of the vibration frequency change in each measurement urea concentration of the urea sensor of this example manufactured by the method mentioned above. It turns out with the increment in the amount of ureas that vibration frequency decreases at a fixed rate so that clearly from the graph of drawing 7 . Moreover, this urea sensor did not have a response in oscillation frequency to the amounts of chemicals other than ureas, such as a glucose. Therefore, it has been checked that property sufficient as an urea sensor in this example had been acquired.

[0056] Next, the measurement result of aging of the output signal at the time of the urea sensor of the 2nd example and the urea sensor of the example of a comparison being immersed in the phosphate buffer solution containing the ten to 3 g/ml amount of ureas at drawing 8 , respectively is shown. The horizontal axis of a graph shows the elapsed time (minute) from measurement initiation, and an axis of ordinate sets variation of oscillation frequency and output potential to 100, and shows the variation in each saturation state for it by the relative value. The curve V in a graph shows aging of the oscillation frequency of the urea sensor of the 2nd example, and the curve III in a graph shows aging of the output voltage of the urea sensor explained in the conventional example as an example of a comparison. In the urea sensor of the 2nd example, the variation of oscillation frequency reached the saturation value mostly within 1 minute to having required for the urea sensor of the example of a comparison for about 20 minutes that the variation of output voltage reaches a saturation value so that clearly from the graph of drawing 5 . Thus, it has checked that the quantum of the amount of the amount of chemicals could be carried out in a short time by the urea sensor which is an example of the enzyme sensor using the quantum method of the amount of chemicals this invention, and it.

[0057] Moreover, the variation of oscillation frequency reached the saturation value mostly within 1 minute similarly [in the urea sensor which fixed and manufactured the urease using the specific binding

of streptoavidin and a biotin / that a glutaraldehyde was used].

[0058] Moreover, although the oscillation frequency of a surface acoustic wave device was measured as an output signal of an urea sensor in the 2nd example mentioned above, the resonance frequency of a surface acoustic wave device may be measured in this invention.

[0059]

[Effect of the Invention] According to this invention, fix an enzyme to vibrator, an enzyme-substrate complex is made to adhere to vibrator by the enzyme reaction, and change of the vibration frequency of this vibrator according to this coating weight is detected. For this reason, if the coating weight of the amount of chemicals to vibrator becomes constant value when an enzyme reaction will be in a steady state, without passing through the process which the product by the amount of chemicals and enzyme reaction which carry out a quantum diffuses, the quantum of the amount of chemicals can be carried out from vibration frequency change of the vibrator in that event. Consequently, as compared with the quantum method of the conventional amount of chemicals, a quantum can be carried out by short time amount.

[0060] Moreover, since the covalent bond of an enzyme and a glutaraldehyde, the specific binding reaction of avidin and a biotin, or the specific binding reaction of streptoavidin and a biotin is used [according to the manufacture method of the enzyme sensor this invention] in fixing an enzyme to a quartz resonator, an enzyme is firmly combinable to a quartz resonator.

[0061] Moreover, this invention uses and is suitable for the quantum of for example, the amount of ureas.

[Translation done.]

* NOTICES *

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1.This document has been translated by computer. So the translation may not reflect the original precisely.

2.**** shows the word which can not be translated.

3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] (A) - (C) is process drawing with which explanation of the urea sensor of the 1st example and its manufacture method is presented.

[Drawing 2] (A) - (C) is process drawing with which explanation of the urea sensor of the 2nd example and its manufacture method is presented.

[Drawing 3] It is the block diagram having shown the system used for measurement of the response characteristic of the urea sensor of the 1st example.

[Drawing 4] It is the graph which shows the measurement result of the response characteristic of the urea sensor of the 1st example.

[Drawing 5] It is the graph which shows the measurement result of aging of the output signal of the urea sensor of the 1st example, and the urea sensor of the example of a comparison.

[Drawing 6] It is the block diagram having shown the system used for measurement of the response characteristic of the urea sensor of the 2nd example.

[Drawing 7] It is the graph which shows the measurement result of the response characteristic of the urea sensor of the 2nd example.

[Drawing 8] It is the graph which shows the measurement result of aging of the output signal of the urea sensor of the 2nd example, and the urea sensor of the example of a comparison.

[Drawing 9] It is the cross section with which explanation of the conventional urea sensor is presented.

[Description of Notations]

12: Porous glass film 14: Urease

24: Silver-silver chloride electrode

26: Ammonia permselective membrane

28: Urea 30: Quartz resonator

34: Biotin

36: Biotin long arm 38: Avidin

40: Urease

42: Urea FET sensor 44: Terminal

46: Oscillator circuit 48: Radionuclide generator

50: Frequency counter 52: Computer

54: Beaker 56: Phosphate buffer solution

60: Urea sensor 62: Amplifier

64: Amplifier 66: Cistern

68: Phosphate buffer solution

70: Surface acoustic wave device 72: Quartz plate

74: Transducer

76: Transducer

[Translation done.]